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SOHO state-of-the-art update and next questions: MPN

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Abstract

The discovery of the activating *JAK2*^{V617F} mutation in 2005 in the majority of patients with the classic Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) spurred intense interest in research into these disorders, culminating in the identification of activating mutations in *MPL* in 2006 and indels in *CALR* in 2013, thus providing additional mechanistic explanations for the universal activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) observed in these conditions, and the success of the JAK1/2 inhibitor ruxolitinib, which first received regulatory approval in 2011. The field has continued to advance rapidly since then, and the last two years have witnessed important changes to the classification of MPN and diagnostic criteria for polycythemia vera (PV), novel insights into the mechanisms of bone marrow fibrosis in primary myelofibrosis (PMF), increasing appreciation of the biologic differences between essential thrombocythemia (ET), prefibrotic and overt PMF and between primary and post-PV/ET myelofibrosis (MF). Additionally, the mechanisms through which mutant calreticulin drives JAK-STAT pathway activation and oncogenic transformation are now better understood. Although mastocytosis is no longer included under the broad heading of MPN in the 2016 revision to the World Health Organization classification, an important milestone in mastocytosis research was reached in 2017 with the regulatory approval of midostaurin for patients with advanced systemic mastocytosis (AdvSM). In this article, we review the major recent developments in the areas of PV, ET and MF, and also briefly summarize the literature on midostaurin and other KIT inhibitors for patients with AdvSM.

Keywords

myeloproliferative neoplasms; polycythemia vera; essential thrombocythemia; myelofibrosis; pre-fibrotic; ruxolitinib; pacritinib; midostaurin

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Introduction

The field of myeloproliferative neoplasms (MPN) has come a long way since their original recognition and description as a group of related disorders in William Dameshek's classic treatise published in 1951.[1] Improvements in our understanding of the genetic underpinnings of many of these conditions, as well as refinements in morphologic classification, have led to the creation of a separate major category for mastocytosis, a biologically unique set of related disorders, and the designation of pre-fibrotic primary myelofibrosis, previously a provisional category under primary myelofibrosis, as a distinct entity.[2] Elucidation of molecular mechanisms has provided new therapeutic insights, such as the successful use of ruxolitinib for rare MPN such as chronic neutrophilic leukemia (CNL)[3] and for the rare myeloid neoplasm associated with t(8;9)(p22;p24.1), that fuses the *PCM1* gene to the *JAK2* gene.[4] The topic of eosinophilia-associated MPN has recently been reviewed.[5] In this paper, we summarize the major recent developments in the classic Philadelphia chromosome-negative (Ph^-) MPN, polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF), as well as provide a brief update on the evolving management of advanced systemic mastocytosis (SM).

Polycythemia vera: state of the art update

Diagnosis

The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms contains significant changes to the diagnostic criteria for PV (Table 1).[2] This stems from the recognition that individuals with so-called "masked" PV have higher rates of progression to MF and inferior leukemia-free survival (LFS) and overall survival (OS) as compared to those with overt PV,[6,7] which may reflect, in part, a lower intensity of therapy in these patients because of missed/delayed diagnosis.[8] Thus, the hemoglobin and hematocrit thresholds for making a diagnosis of PV in men and women have been lowered to 16.5 g/dl/0.49 and 16 g/dl/0.48, respectively.[2] Some experts continue to use Cr^{51}-red cell mass (RCM) measurements to diagnose PV, especially in patients who do not meet the 2008 WHO criteria for elevated hemoglobin or hematocrit values (>18.5 g/dl/0.6 in men and >16.5 g/dl/0.56 in women),[9] pointing out that the hematocrit is a derived value sensitive to changes in red cell size;[10] however, RCM measurement is not readily available, even at most tertiary care centers. The second major change to the diagnostic criteria for PV concerns the role of bone marrow biopsy. This is now a requirement for diagnosis, except in obvious cases that meet the higher WHO 2008 cutoffs for hemoglobin and hematocrit.[2] The enhanced emphasis on bone marrow examination was based on the need to distinguish PV morphologically from cases of *JAK2*-mutated ET with polycythemia in the context of the lowered hemoglobin and hematocrit thresholds for PV diagnosis.[11,12]

Therapy: the resurgence of interferons

Hydroxyurea (HU) is currently the most commonly used first-line drug in patients with PV who require cytoreduction.[13] The use of ruxolitinib as second-line therapy after failure of HU (Table 2)[14] is supported by two large, randomized controlled trials (RCTs), RESPONSE[15,16] and RESPONSE-2.[17] Interferons, which are distinguished by their ability

to induce molecular remissions,[18,19] may also be used as initial therapy, and are increasingly preferred for young patients given their lack of leukemogenicity.[20,21] Long-term follow-up of a single-institution trial of pegylated interferon alfa (IFN-α), however, shows that discontinuation rates due to toxicity can be substantial.[22] Ropeginterferon alfa-2b is a new monopegylated isoform of IFN-α-2b that has the advantage of requiring administration only every 2 weeks. This agent, which produced an overall response rate (ORR) of 90% (47% complete, 43% partial) in the phase 1/2 PEGINVERA study[23] with a complete molecular response (CMR) in 21% of patients and a partial molecular response in 47% of patients, was subsequently compared head-to-head with HU in the phase 3 PROUD-PV RCT.[24] Non-inferiority in the 12-month complete hematologic response (CHR) rate was demonstrated in this trial, which enrolled both cytoreductive treatment-naïve patients and those who had been on HU for <3 years without achievement of CHR or development of resistance or intolerance.[24] Pegylated IFN-α-2a (Pegasys®) is also being compared to HU in the frontline setting in a large multi-center phase 3 RCT.[25] A planned interim analysis of this trial performed after 75 patients had been on study for 12 months did not show a statistically significant difference in ORR between the two arms (69% for HU and 81% for pegylated IFN-α-2a).[25]

PV: areas of controversy and next questions

Although *JAK2*^{V617F} expression is sufficient to produce the PV phenotype in mouse models,[26–29] several lines of evidence suggest that additional genetic and/or epigenetic lesions are necessary to sustain the disease.[30,31] Patients who acquire *JAK2*^{V617F} before a *TET2* mutation are more likely to present with PV than ET, and have an increased risk of thrombosis.[32] Homozygosity for *JAK2*^{V617F}, strongly correlated with a PV phenotype, is more common in men.[33] However, even after removing gender as a potential confounder, gene expression profiling reveals two distinct clinical phenotypes in PV.[34] In one series of 133 patients with PV, targeted sequencing revealed one or more sequence variants/mutations (in genes other than *JAK2*) in 53%; variants/mutations in *ASXL1, SRSF2* and *IDH2* (combined prevalence 15%) were identified as being prognostically adverse.[35] Clearly, there are factors beyond canonical JAK-STAT signaling that are involved in the pathogenesis and maintenance of PV (and other MPN); these are yet to be clearly elucidated. Thrombotic risk stratification in PV continues to rely on two variables: patient age and previous history of thrombosis.[21] Whether or not leukocytosis contributes to the risk of thrombosis is still controversial, with some studies finding leukocytosis to independently predict thrombosis,[36–38] and others reporting no association;[39] furthermore, the precise cutoffs are not clear. Leukocytosis ≥15 × 10^9/L was found to adversely affect both OS and LFS in a large (n = 1,545) study.[40] While some evidence suggests that control of leukocytosis with cytoreductive therapy may decrease the risk of thrombosis,[41] this remains debatable, and isolation of the effect of leukocytosis reduction from that of hematocrit control is difficult.

An interesting new strategy that has entered the clinic in both high-risk, previously treated PV and *JAK2*$^{V617F+}$ ET is that of using small-molecule inhibitors of human double minute 2 (MDM2), which trigger p53-dependent apoptosis in the absence of deletions or inactivating mutations of *TP53* (NCT02407080). Based on preclinical studies showing synergism,[42,43] pegylated IFN-α can be added in cases of insufficient response. While an attractive

laboratory-based concept, whether this class of drugs could gain regulatory approval in these indolent diseases, given their expense and toxicity and the approval of ruxolitinib for second-line therapy of PV and ongoing development for ET (see below) is questionable. Similar considerations apply to histone deacetylase inhibitors (reviewed in ref.[44]), which are clinically active but have chronic toxicities.

Essential thrombocythemia: state of the art update

CALR mutations: pathophysiology and clinical correlations in ET

In 2013, mutations in exon 9 of the gene encoding calreticulin (CALR), an endoplasmic reticulum (ER) chaperone, were reported in 20–30% of patients with ET and primary MF (PMF), accounting for the majority of *JAK2/MPL*-unmutated cases.[45,46] These mutations, that can broadly be divided into 52-base pair deletions (termed type 1/type-1 like) or 5-base pair insertions (termed type 2/type 2-like), all result in an altered C-terminus of the mutant protein with loss of negative charge and impaired Ca^{++} binding, loss of the "KDEL" ER retention motif, and activation of the Janus kinase – signal transducer and activator of transcription (JAK-STAT) pathway.[45,46] Recent work from several groups has demonstrated that mutant CALR must bind to and activate MPL (the thrombopoietin receptor) to drive MPN pathogenesis, and that the altered C-terminus is required for oncogenic transformation.[47–50] *CALR* mutations appear to confer a greater proliferative advantage to the neoplastic clone compared with *JAK2* mutations; clonal expansion is faster in *CALR*-mutated cases than in *JAK2*-mutated cases, both in ET and in PMF.[51]

CALR-mutated patients with ET tend to be younger, more frequently male, and have higher platelet counts and lower hemoglobin levels and leukocyte counts than their *JAK2*-mutated counterparts.[52–54] Of particular importance, thrombotic risk in *CALR*-mutant ET appears particularly low, so much so that young patients with CALR-mutated ET and no history of thrombosis (i.e., "very low risk" patients) may forego aspirin.[20,55,56] Most studies have found no impact of *CALR* mutations on OS, leukemic transformation (LT) or the risk of progression of ET to MF,[52–54] although it was recently reported that *CALR*-mutated ET patients progress more slowly to MF than *JAK2*-mutated or triple negative patients.[57] Type 1/type 1-like and type 2/type 2-like *CALR* mutations occur at approximately equal frequencies in ET, and although one study noted a significantly higher risk of progression to MF among ET patients with type 1/type 1-like *CALR* mutations as opposed to type 2/type 2-like mutations,[58] other investigators have not found this to be the case.[59]

Advances in risk stratification and current and future therapy of ET

ET is the most indolent of the classic MPN,[60,61] and treatment is currently based on thrombotic risk.[21] The latter is estimated using the revised IPSET (International Prognostic Score for ET)-thrombosis score (Table 3), which takes into account patient age, thrombosis history and presence or absence of $JAK2^{V617F}$ and cardiovascular risk factors.[56] This model has been validated in an independent cohort,[62] and is not impacted by *CALR* mutational status.[63] Importantly, leukocytosis $\geq 11 \times 10^9$/L, which is not a prognostic variable in the IPSET-thrombosis model, is, however, predictive of worse survival in ET, as are advanced age (≥60 years) and prior thrombosis.[64] Furthermore, a number of studies have reported a

correlation between leukocytosis, but not thrombocytosis, with the risk of thrombosis in ET.[65–68] Very recently, a two-center study of 1,494 patients found male sex, age ≥60 years and leukocyte count ≥11 × 10^9/L to be independent predictors of shortened survival in ET; thrombosis history was not significant upon multivariate analysis in this study.[69] An increased serum lactate dehydrogenase level has also been reported to correlate with inferior survival in ET,[70] which leads one to speculate whether these patients might, in fact, have had pre-PMF (discussed below).

HU is usually the preferred agent for cytoreduction in ET for patients who need cytoreductive therapy, based on the findings of the PT-1 study, in which HU was compared head-to-head with anagrelide.[71] More recently, anagrelide was found to be non-inferior to HU in the ANAHYDRET study;[72] nevertheless, this agent is typically used second-line.[21] It is important to appreciate that the patient populations studied in the PT-1 and ANAHYDRET studies and their designs were not identical; the definition of ET relied on different criteria (Polycythemia Vera Study Group (PVSG) criteria in PT-1 and WHO in ANAHYDRET), the patients in PT-1 could have received prior therapy, whereas those in ANAHYDRET could not, and the use of aspirin was not mandated in the ANAHYDRET study. As in PV, interferons may be preferred over HU in young patients.[21] Formal criteria to define resistance and intolerance to HU in patients with ET have been published (Table 4).[73] Not captured in these criteria are the substantial symptom burden that patients with ET can have,[74,75] which may not be alleviated by standard cytoreductive therapies even in the presence of hematologic response. In a phase 1/2 study in 39 patients with HU-resistant/intolerant ET, median platelet and leukocyte counts decreased rapidly with ruxolitinib over the first 4 weeks of therapy, and many patients experienced a ≥50% improvement in a variety of symptoms by week 12.[76] However, ruxolitinib did not improve the rate of CHR compared with best available therapy (BAT) in HU-resistant/intolerant ET patients in the MAJIC randomized clinical trial; rates of thrombosis, hemorrhage or progression to MF did not significantly differ, either.[77] Nevertheless, ruxolitinib is currently being tested as second-line therapy in patients with high risk ET in two trials: the RESET-272 study (NCT03123588) in the US in which it is compared to anagrelide, and in a French study (NCT02962388) in which it is compared to anagrelide or IFN-α. The RUXO-BEAT trial (NCT02577926) in Germany compares ruxolitinib to BAT in patients with high-risk ET who may be treatment-naïve or previously treated; patients with high risk PV with prior exposure to cytoreductive agents for ≤6 weeks may also enroll.

ET: areas of controversy and next questions

Although *JAK2*^{V617F} is classically associated with a PV phenotype,[26–29] mice expressing this mutation have been shown to develop ET-, PMF- and PV-like disease, and differences in gene dosage/mutant allele burden have been invoked as explanations of how the same mutation can lead to different disease phenotypes.[78,79] Indeed, knock-in mouse models of ET with variable rates of progression to MF using all 3 driver mutations (*JAK2*^{V617F}, *MPL*^{W515L} and *CALR* exon 9 mutations) have been generated.[50,79–82] Furthermore, as noted above, mutation order influences disease phenotype in the Ph$^-$ MPN: prior mutation of *TET2* has been shown to alter the transcriptional signature of *JAK2*^{V617F} in a cell-intrinsic manner, preventing the latter from up-regulating genes associated with proliferation.[32]

Experimentally, it has been shown in a mouse model that loss of STAT1 in the presence of *JAK2*^{V617F} promotes a PV phenotype over an ET phenotype, while activating STAT1 using IFN-γ has the opposite effect.[83] Targeted deep sequencing of 183 patients with ET revealed the presence of one or more sequence variants/mutations in "non-driver" genes in 53%; those in *SH2B3, SF3B1, U2AF1, TP53, IDH2* and *EZH2* (combined prevalence 15%) adversely impacted survival.[35] As the mutational landscape of ET and PV is further unraveled, new insights into disease pathogenesis are likely to emerge.

In the clinical setting, although considerable evidence points to a higher risk of thrombosis[65–68] and worse survival[64] in patients with leukocytosis, the cutoffs vary across studies and it remains unknown if control of leukocytosis with cytoreductive therapy will improve outcomes, although some experts emphasize this in their practices.[84] At present, leukocytosis is not routinely considered when making therapeutic decisions, which continue to be informed by thrombotic risk, as determined by the revised IPSET-thrombosis score.[56] In the case of thrombocytosis, there is no good evidence of a correlation between platelet count and clotting risk; rather, most physicians check for the presence of acquired von Willebrand disease and use cytoreductive drugs to mitigate bleeding risk in patients with "extreme thrombocytosis" (reviewed in ref.[85]).

Myelofibrosis: state of the art update

Pre-fibrotic primary myelofibrosis

The 2016 WHO classification of myeloid neoplasms recognizes pre-fibrotic PMF (pre-PMF) as a separate entity under the MPN umbrella, an important change from prior versions that included pre-PMF as a "provisional" category within PMF.[2] Diagnostic criteria for pre-PMF appear in Table 5, and the pathologic distinction between ET and pre-PMF has been the subject of considerable controversy over the years.[86] Nevertheless, it is clear that compared to patients with ET, patients with pre-PMF have worse OS and higher rates of LT and progression to overt MF.[87–89] Patients with pre-PMF also appear to have higher rates of thrombosis[90] and bleeding[91] than those with ET and, as in ET, leukocytosis may predict for an increased risk of thrombosis, arterial in particular, among patients with pre-PMF.[92,93] Recently, the findings from two large studies totaling over 1000 patients with PMF support the existence of a phenotypic continuum from pre-fibrotic to overt PMF, with overt PMF being associated with a higher incidence of cytopenias, higher circulating blast counts, greater symptom burden and higher incidence of splenomegaly, as well as worse prognostic risk scores and significantly reduced OS compared with pre-PMF.[94,95]

Novel insights into mechanisms of bone marrow fibrosis in PMF

PMF is distinguished clinically from PV and ET by the development of anemia in nearly all patients, a much higher incidence of splenomegaly and a significantly greater symptom burden, along with a substantially higher risk of LT and markedly shortened survival.[96] The intrinsic biologic complexity of PMF is considerably greater than that of PV or ET; this is reflected in the mutational burden of the three diseases.[97] In fact, PMF has been proposed to be best considered a myelodysplastic/myeloproliferative neoplasm, characterized by a high frequency of "non-driver" mutations affecting epigenetic regulation and the spliceosome

machinery.[98] Bone marrow fibrosis in PMF has classically been viewed as being a reactive process.[10] However, some recent findings have challenged this notion. For example, it has been demonstrated that bone marrow from patients with PMF is rich in clonal, neoplastic monocyte-derived fibrocytes that produce collagen and fibronectin and give rise to a lethal MF-like phenotype when transplanted into immunodeficient mice.[99] Other investigators have shown that $Gli1^+$ mesenchymal stromal cells (MSCs) are recruited from endosteal and perivascular niches to become fibrosis-driving myofibroblasts in *JAK2*$^{V617F+}$ mouse models and in the bone marrow of MPN patients,[100] and proposed that non-canonical modes of activation of the Gli transcription factors may explain the modest effects of therapeutic hedgehog (smoothened) inhibition in MF.[101] Overexpression of v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF) has recently been implicated in the pathogenesis of bone marrow fibrosis in PMF through excessive production of the pro-fibrotic mediator SPP1 and resultant proliferation of fibroblasts and MSCs, leading to collagen production.[102]

Improvements in prognostication

Although many studies evaluating prognostic factors in patients with PMF have been published over the years, the most frequently used prognostic scoring systems in clinical practice are the International Prognostic Scoring System (IPSS), the Dynamic IPSS (DIPSS) and the DIPSS-plus, which incorporates karyotype together with clinical variables (reviewed in[103]). Circulating or bone marrow blasts ≥10%, platelets $<50 \times 10^9$/L and chromosome 17 aberrations have been found to characterize an "accelerated phase" in patients with MF, which appears to be a necessary step in LT of chronic phase MF and portends an extremely poor prognosis.[104] Other investigators have reported >80% 2-year mortality in PMF patients with monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of the following: circulating blasts ≥10%, leukocyte count $\geq 40 \times 10^9$/L, or other unfavorable karyotype.[105] The demonstration that PMF patients with CALR mutations have the best OS, and so-called "triple negative" patients exhibit the worst OS,[106,107] as well as the identification of "high molecular risk" (HMR) mutations in PMF (*ASXL1, EZH2, IDH1/2, SRSF2*),[108,109] has led to efforts to incorporate mutational data into prognostic models for PMF.[110] Others have proposed prognostic scoring systems that only take into account age and genomic information.[111,112] Unlike in ET, type 1/type 1-like and type 2/type 2-like *CALR* mutations have been shown to have different prognostic impacts, with the favorable prognosis associated with *CALR* mutations restricted to the more common type 1 mutations.[113,114] Marked separation of the survival curves of PMF patients stratified only by the mutational status of *CALR* and *ASXL1* has also been reported.[115] Although not included in the major prognostic models, the grade of bone marrow fibrosis (0–1 versus 2–3) was recently shown to significantly impact OS, independent of IPSS variables and mutational status, with patients with higher grades of fibrosis also being more likely to have cytopenias, constitutional symptoms, larger splenomegaly and HMR mutations.[116] Lastly, thrombotic risk in PMF appears largely restricted to patients with *JAK2* mutations.[117]

Progression of PV to MF occurs in 4.9–6% of cases at 10 years and 6–14% at 15 years; the corresponding percentages for ET are 0.8–4.9% and 4–11%, respectively.[118] A variety of risk factors for progression to post-PV/ET MF have been identified (reviewed in ref.[118]).

Although managed similarly,[119] a number of groups have reported significant differences between the clinical behavior of PMF and post-PV/ET MF, with the latter representing a more indolent disease process characterized by better survival, more so for post-ET MF than for post-PV MF, and found that prognostic models developed from cohorts of patients with PMF, such as the IPSS, DIPSS and DIPSS-plus, do not reliably distinguish between prognostic categories in post-PV/ET MF.[120–123] Prior studies in small numbers of patients had identified anemia (hemoglobin <10 g/dL), thrombocytopenia (platelets $<100 \times 10^9$/L) and leukocytosis (WBCs $>30 \times 10^9$/L) as being prognostically adverse in post-PV MF,[124] and unfavorable karyotype in both post-PV and post-ET MF.[125] These observations laid the foundation for the large (n = 781) Myelofibrosis Secondary to PV or ET (MYSEC) project, the results of which were recently published.[126] The superior survival of patients with post-ET MF compared with those with post-PV MF was confirmed in this study (median, 14.5 versus 8.1 years), as was that of *CALR*-mutated patients when compared to *JAK2*-mutated patients, as is the case in PMF.[57,126] There was no difference in terms of OS between patients with type 1/type-1 like and type 2/type-2 like *CALR* mutations.[57] Among patients with post-ET MF, rates of LT were significantly higher among those with triple negative or *JAK2*-mutated disease than those with *CALR*-mutated disease, while thrombosis risk was not affected by driver mutation status.[57] The researchers identified five variables (Table 6) which, when combined with patient age on a nomogram in a prognostic model (the MYSEC-PM), allow allocation to one of four prognostic categories (low, intermediate-1, intermediate-2 and high risk) with significantly different survival times (median, not reached, 9.3, 4.4 and 2 years, respectively).[126]

Update on ruxolitinib

Six years after its approval,[127] ruxolitinib remains the only approved agent for the treatment of MF. The final, 5-year updates of the pivotal COMFORT trials were recently published.[128,129] Overall, the rates of best response improved over time, no new safety signals emerged, and the median duration of spleen response was about 3 years. Although these trials were not powered for survival, patients originally assigned to ruxolitinib lived longer than those assigned to placebo or BAT, despite near-complete crossover, confirming the superior survival observed at earlier timepoints.[130–132] The rates of reduction in bone marrow fibrosis grade and mutant *JAK2* allele burden remain modest.[128,129] Interestingly, comparing across trials and diseases, mutant *JAK2* allele burden reduction with ruxolitinib appears more robust in the RESPONSE trial in PV.[133] In the COMFORT-1 trial, greater reductions in the mutant *JAK2* allele burden occurred in patients with shorter disease duration, potentially arguing for the use of ruxolitinib in less advanced stages of the disease.[134] A substantial amount of data supports the use of ruxolitinib in patients with IPSS intermediate-1 risk disease (reviewed in ref.[135]). The phase 3, placebo-controlled ReTHINK trial, designed to evaluate ruxolitinib in patients with lower risk disease with one or more HMR mutations but no significant symptoms or splenomegaly, had to be closed due to poor accrual.[136] Current consensus guidelines recommend the use of ruxolitinib in low risk patients with troublesome symptoms and/or splenomegaly,[137] but not for its survival advantage, citing “weak evidence”.[138]

Other than a study that reported a greater benefit of ruxolitinib in patients with a *JAK2*^{V617F} allele burden ≥50%,[139] no factors have been identified that predict its clinical efficacy in patients with MF.[140] Several key insights have been provided by analyses of the ~100 patients enrolled on the phase 1/2 trial of ruxolitinib[141] at the MD Anderson Cancer Center. Spleen responses appear dose-dependent and correlate with survival,[142] a finding confirmed in a pooled analysis of the COMFORT trials.[143] Furthermore, the presence of ≥3 non-driver mutations, mainly affecting epigenetic regulators, is associated with much lower odds of having a spleen response, a shorter time to treatment discontinuation and inferior survival.[144] Finally, among patients who discontinued ruxolitinib, declining platelet counts and clonal evolution on ruxolitinib therapy predicted for worse outcomes.[145] Anemia, an on-target phenomenon resulting from JAK2 inhibition, often impairs dose optimization of ruxolitinib in clinical practice, and is frequently a cause of premature discontinuation. Ruxolitinib-induced anemia is most pronounced during the first 12–24 weeks of therapy, after which hemoglobin levels return to a new, lower baseline. Importantly, it has been shown that ruxolitinib-induced anemia does not share the adverse prognosis of disease-associated anemia[146] and, in fact, that ruxolitinib therapy can overcome the latter.[147] Ruxolitinib should be dosed according to platelet counts as outlined in the prescribing information, along with supportive measures for the anemia (discussed further below).

Novel therapeutic strategies

Anemia remains a significant clinical problem in MF, often hindering dose optimization of ruxolitinib. Currently available therapeutic options, i.e., androgens, steroids, erythropoiesis-stimulating agents (ESAs) and immunomodulatory agents (Imids) are unsatisfactory.[148] An interesting new class of drugs (“activin receptor type II ligand traps”) consists of fusion proteins that sequester ligands belonging to the transforming growth factor beta (TGF-β) superfamily, thereby abrogating their suppressive effect on terminal erythropoiesis.[149] Response rates of ~40% have been reported with sotatercept,[150] the first molecule in this class, and evaluation of this agent is ongoing, both alone and in combination with ruxolitinib (NCT01712308). A clinical trial of the related agent luspatercept in anemic patients with MF will soon open to accrual (NCT03194542). The inhibitor-of-apoptosis (IAP) antagonist LCL-161 has also been shown to produce clinical improvement (CI) in anemia,[151] but this drug is difficult to combine with ruxolitinib on theoretical grounds because of suppression by ruxolitinib of tumor necrosis factor alfa (TNF-α), believed to be necessary for the biological effect of LCL-161[152]. The immunomodulatory agents lenalidomide and pomalidomide, while producing anemia responses in 20–30% of patients when administered alone, can be quite myelosuppressive when administered in conjunction with ruxolitinib.[153,154] On the other hand, thalidomide is relatively non-myelosuppressive and well-tolerated at low doses (i.e., 50 mg/d).[155–157] The combination of ruxolitinib and thalidomide is currently being explored in a clinical trial (NCT03069326) that enrolls both ruxolitinib-naïve patients and those who have had an insufficient response to ruxolitinib.

A number of JAK2 inhibitors besides ruxolitinib have been tested in patients with MF but, unfortunately, none has been approved to date (reviewed in ref.[158]). Most were discontinued because of toxicity; of these, fedratinib, which also inhibits bromodomain extra-terminal (BET) proteins,[159] was in the most advanced phase of clinical development.[160] Development

of this clearly active drug (36–40% spleen volume reduction (SVR) and 34–36% symptom response rates at 24 weeks in JAK inhibitor-naïve patients and a 55% SVR rate in ruxolitinib-exposed patients) was halted due to the occurrence of several cases of suspected Wernicke's encephalopathy.[160,161] Very recently, the development of momelotinib, a JAK1/2 inhibitor that had the unique benefit of improving anemia in patients with MF, was stopped given disappointing results in the SIMPLIFY-1 and SIMPLIFY-2 phase 3 trials in terms of the conventional endpoints of ≥35% SVR and ≥50% reduction in total symptom score (TSS), despite evidence of benefit in anemia-related endpoints.[162,163] The JAK2-selective inhibitor pacritinib was superior to BAT (excluding ruxolitinib) in JAK inhibitor-naïve patients in the phase 3 PERSIST-1 trial,[164] with somewhat mixed results obtained in another phase 3 randomized trial (PERSIST-2) comparing two doses of pacritinib, 400 mg daily and 200 mg twice daily, against BAT in thrombocytopenic patients (baseline platelets $<100 \times 10^9$/L) with MF. Of note, the BAT arm included 40–45% of patients who previously had received ruxolitinib, and 44% of patients assigned to the BAT arm received ruxolitinib at some point during the study.[165] The primary objective was to compare the efficacy of pacritinib, pooling both the dosing arms, to BAT, and the secondary objectives were to compare twice daily and once daily pacritinib individually to BAT. In the primary comparison, 18% of pooled pacritinib patients versus 3% of BAT patients achieved ≥35% SVR (p=0.001), while for ≥50% TSS reduction, these proportions were 25% and 14%, respectively (p=0.079). In the secondary analyses, twice daily pacritinib beat BAT in terms of both ≥35% SVR (22% versus 3%, p=0.001) and ≥50% TSS reduction (32% versus 14%, p=0.011). Once daily pacritinib was superior to BAT for ≥35% SVR (15% versus 3%, p=0.017) but not for ≥50% TSS reduction (17% versus 14%, p=0.652). More patients receiving pacritinib than BAT experienced a ≥50% reduction in their red blood cell transfusion burden. Both gastrointestinal and hematologic adverse events were generally less frequent in the twice daily dosing group. Concerns over excess mortality in the pacritinib-treated patients in these trials prompted the Food and Drug Administration (FDA) to mandate a dose-finding study (PAC203, NCT03165734) in thrombocytopenic patients with MF failing ruxolitinib. Preliminary results from a phase 2 study of another JAK2-selective inhibitor, NS-018, in ruxolitinib-pretreated patients showed a ≥35% SVR rate of 12% and a ≥50% TSS improvement rate of 35%.[166] Finally, the JAK1 inhibitor itacitinib yielded encouraging symptom responses (≥50% TSS reduction in 30–35% of patients at 12–24 weeks) in a phase 2 trial;[167] this agent is now being studied in combination with low-dose ruxolitinib, as well as alone in patients who fail ruxolitinib after initial response (NCT03144687).

A plethora of novel, targeted agents are under study in patients with MF, either alone or in combination with ruxolitinib (for recent reviews of this subject, see refs.[168,169]). The telomerase inhibitor imetelstat generated much enthusiasm after complete and partial remissions, reversal of bone marrow fibrosis and molecular responses were reported in a pilot study,[170] but this was substantially dampened by updates from the IMBARK™ study in JAK inhibitor-exposed patients with relapsed/refractory intermediate-2/high risk MF. Enrollment to the lower-dose arm has been suspended, and an efficacy and safety analysis of the enrolled higher-dose arm is currently ongoing.[171] The anti-fibrotic agent PRM-151 (recombinant pentraxin-2) slowed the development of bone marrow fibrosis *in vivo* and

prolonged the survival of immunodeficient mice transplanted with bone marrow cells from patients with MF.[99] Promising findings were presented from a clinical trial, both in patients receiving PRM-151 alone and in combination with ruxolitinib.[172,173] Enrollment in a pivotal trial of PRM-151 (NCT01981850) has since been completed, and results are expected soon. Many studies evaluating ruxolitinib in combination with other targeted agents are underway (Table 7); some of these, e.g., those combining ruxolitinib with inhibitors of histone deacetylases, heat shock protein 90 (HSP90) and phosphatidylinositol-3-kinase (PI3K) are backed by sound preclinical data.[174–179] Thus far, the only combinations that have appeared to yield somewhat better responses than expected with ruxolitinib alone have been those with azacitidine[180] and panobinostat.[181] Clinical data are awaited on other concepts supported by compelling preclinical findings, e.g., the combination of ruxolitinib with the cyclin-dependent kinase 4/6 inhibitor ribociclib and a PIM kinase inhibitor.[182] Yet other novel drug classes, e.g., BH3-mimetics,[183] selective inhibitors of nuclear transport[184] and BET inhibitors/proteolysis-targeting chimeras (PROTACs)[185,186] appear highly promising in the laboratory but are yet to enter the clinic in patients with MF.

Myelofibrosis: next questions

Whether or not ruxolitinib improves OS in patients with MF continues to be debated, with concerns raised over the COMFORT trials not being powered to show differences in survival and comparisons with historical controls being flawed due to imbalances in patient characteristics.[187] Some experts have suggested that the survival advantage for ruxolitinib observed in the COMFORT trials may reflect improvements in appetite, weight and overall functionality rather than a true disease-modifying effect.[188] Indeed, the limited effects of ruxolitinib on bone marrow fibrosis and driver mutation allele burden suggest that any disease-modifying activity of the drug is likely to be relatively minor. The ReTHINK trial[136] sought to answer this question by evaluating the drug in patients with genetically high-risk disease without significant splenomegaly or symptoms, but had to be closed owing to poor accrual. Rational, ruxolitinib-based combinations may be the way forward, but thus far, no clear winner has emerged among the combinations for which clinical data is available. Similarly, the pathogenesis of bone marrow fibrosis remains poorly understood, and although many agents have been investigated, there are no drugs available at present that convincingly improve bone marrow fibrosis in MF.

Mechanisms of resistance to ruxolitinib remain unclear. It has been shown preclinically that MF is intrinsically more resistant to JAK2 inhibition than PV or ET.[189] Although resistance-conferring mutations in the kinase domain of JAK2 have been described,[190] these are rare and not clinically relevant in most patients. JAK2 inhibitor “persistence” has been described as a mechanism of therapeutic resistance to conventional (type 1) JAK2 inhibitors,[191] and has been shown to be overcome by drugs that degrade JAK2, such as HSP90 inhibitors,[176] or by “type 2” JAK2 inhibitors that bind to and stabilize the kinase in its inactive conformation;[192] however, no such agent is in clinical trials yet. From a clinical drug development perspective, there continues to be a major unmet need for a JAK2 inhibitor that is effective after ruxolitinib failure and/or that can be used safely in severely thrombocytopenic (platelets $<50 \times 10^9$/L) subjects. Whether pacritinib or NS-018 will fulfill this need remains to be seen. The future role, if any, of JAK1-selective inhibitors such as

itacitinib is unclear at this time, given the understandably low rate of SVR. Equally, the data available in the public domain at this time do not suggest that any of the other classes of drugs used as single agents, e.g., imetelstat, PRM-151 or LCL-161 are close to regulatory approval. Finally, the quest for an effective agent for MF-associated anemia, particularly for use in conjunction with ruxolitinib, continues. Enrollment of patients on clinical trials continues to be of paramount importance.

Recent developments in advanced systemic mastocytosis

The multi-kinase inhibitor, midostaurin, was recently approved by the FDA for the treatment of patients with aggressive systemic mastocytosis (ASM), systemic mastocytosis with an antecedent hematologic neoplasm (SM-AHN) and mast cell leukemia (MCL) based on the findings of a single-arm non-comparative trial (n = 116) in which midostaurin produced an ORR of 60% with a major response (complete resolution of at least one type of SM-related organ damage) in 45% of patients.[193] The median OS was 28.7 months, and the median progression-free survival, 14.1 months. As midostaurin also inhibits fms-like tyrosine kinase 3 (FLT3), the most common toxicities were gastrointestinal in nature.[193] Midostaurin was effective regardless of the presence or absence of KIT^{D816V}, advanced SM subtype, or exposure to prior therapies. Although SM is a mutant *KIT*-driven disorder, most patients with advanced SM (especially patients with SM-AHN) harbor additional mutations.[194] Mutations in *SRSF2, ASXL1* and *RUNX1* (*S/A/R*) have recently been identified as being associated with inferior OS in advanced SM, as has the number of mutated genes in the so-called *S/A/R* panel.[195,196] Furthermore, these and other mutations (e.g., *TET2*) have been shown to precede the acquisition of KIT^{D816V}.[197] Mutational profiling of patients receiving midostaurin on the aforementioned pivotal trial showed that reduction of the KIT^{D816V} allele burden by ≥25% at six months predicted for improved OS, while the $S/A/R^{+}$ genotype and clonal evolution on midostaurin were associated with worse survival and disease progression, respectively.[198] The success of midostaurin has spurred the development of other KIT inhibitors for patients with advanced SM. BLU-285 is a potent and highly selective inhibitor of *KIT* exon 17 mutants, including the D816V mutant found in >80% of patients with SM;[199] it also inhibits the common D842V mutant of platelet-derived growth factor receptor alfa (PDGFRA).[200] Promising data from an ongoing phase 1 trial (NCT02561988) of this agent in patients with advanced SM have been presented, with improvements in symptoms and C-findings, as well as in objective measures of mast cell burden.[201] DCC-2618 is a potent pan-KIT and PDGFR “switch control” inhibitor that is also being studied (NCT02571036) in patients with advanced SM;[202] clinical data with this agent are not available yet.

Conclusion

These are exciting times in MPN research. In just the last two years, the field has witnessed major changes such as important modifications to the WHO diagnostic criteria for PV, elucidation of how *CALR* mutations activate the JAK-STAT pathway in ET and PMF, establishment of a prognostic scoring system specifically for patients with post-PV/ET MF, and the first-ever drug approval for patients with advanced SM. These achievements have been the result of both astute clinical observations and elegant preclinical work. There

remain, however, many unanswered questions regarding the biology of MPN and major unmet clinical needs. Hopefully, the coming years will see an even more accelerated pace of discovery for the benefit of our patients.

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Table 1

2016 World Health Organization (WHO) Criteria for the diagnosis of polycythemia vera[2]

Major criteria
1. Hemoglobin >16.5 g/dL in men and >16 g/dL in women or, Hematocrit >49% in men and >48% in women or, red cell mass (RCM) >25% above mean normal predicted value
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleiomorphic, mature megakaryocytes (differences in size)
3. Presence of a *JAK2*^{V617F} or *JAK2* exon 12 mutation
Minor criterion
Subnormal erythropoietin level

Diagnosis requires meeting all three major criteria, or the first two major criteria and the minor criterion. Bone marrow biopsy may not be required in cases with sustained absolute erythrocytosis (hemoglobin levels >18.5 g/dL in men (hematocrit >55.5%) and >16.5 g/dL in women (hematocrit >49.5%) if major criterion 3 and the minor criterion are present.

Table 2

ELN definition of resistance and intolerance to hydroxyurea in polycythemia vera[14]

1. Need for phlebotomy to keep hematocrit <45% after 3 months of ≥2 g/day of HU, or
2. Uncontrolled myeloproliferation, i.e., platelets >400 × 10^9/L AND leukocytes >10 × 10^9/L after 3 months of ≥2 g/day of HU, or
3. Failure to reduce massive (≥10 cm below the left costal margin) splenomegaly by ≥50% as measured by palpation, OR failure to completely relieve symptoms related to splenomegaly, after 3 months of ≥2 g/day of HU, or
4. ANC <1 × 10^9/L OR platelets <100 × 10^9/L OR hemoglobin <10 g/dL at the lowest dose of HU required to achieve a complete or partial response (defined below)
5. Presence of leg ulcers or other unacceptable HU-related non-hematologic toxicities, such as mucocutaenous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of HU

Complete response: hematocrit <45% without phlebotomy, platelets <400 × 109/L, leukocytes ≤10 × 109/L, and no disease-related symptoms.

Partial response: hematocrit <45% without phlebotomy, or response in ≥3 other ELN consensus criteria.[203] HU, hydroxyurea; ANC, absolute neutrophil count; ELN, European LeukemiaNet.

Table 3

Revised International Prognostic Score for Thrombosis in Essential Thrombocythemia[56]

Very low risk	No thrombosis history, age ≤60 years, and *JAK2* wild type
Low risk	No thrombosis history, age ≤60 years, and *JAK2* mutation
Intermediate risk	No thrombosis history, age >60 years, and *JAK2* wild type
High risk	Thrombosis history or age >60 years with *JAK2* mutation

Table 4

Definition of resistance and intolerance to hydroxyurea in essential thrombocythemia[73]

1. Platelets >600 × 10^9/L after 3 months of ≥2 g/day of HU (≥2.5 g/day if body weight >80 kg)
2. Platelets <400 × 10^9/L and leukocytes <2.5 × 10^9/L at any dose of HU
3. Platelets <400 × 10^9/L and hemoglobin <10 g/dL at any dose of HU
4. Presence of leg ulcers or other unacceptable muco-cutaneous manifestations at any dose of HU
5. HU-related fever

Table 5

2016 World Health Organization (WHO) criteria for the diagnosis of pre-fibrotic primary myelofibrosis[2]

Major criteria
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often, decreased erythropoiesis
2. Not meeting WHO criteria for CML, PV, ET, MDS, or other myeloid neoplasms
3. Presence of *JAK2, CALR,* or *MPL* mutation or, in the absence of these mutations, presence of another clonal marker, e.g., mutations in *ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, SF3B1,* or absence of minor reactive bone marrow reticulin fibrosis
Minor criteria
1. Anemia not attributed to a comorbid condition
2. Leukocytosis $\geq 11 \times 10^9$/L
3. Palpable splenomegaly
4. LDH above institutional upper limit of normal

Diagnosis requires meeting all three major criteria and at least one minor criterion. CML, chronic myeloid leukemia; PV, polycythemia vera; ET, essential thrombocythemia; MDS, myelodysplastic syndromes.

Table 6

The Myelofibrosis Secondary to PV and ET - Prognostic Model (MYSEC-PM)[126]

Clinical variable	Points assigned
Hemoglobin <11 g/dL	2
Circulating blasts ≥3%	2
CALR-unmutated genotype	2
Platelets <150 × 10^9/L	1
Constitutional symptoms	1

Points total to be used along with patient age on published nomogram to identify risk category.

Table 7

Ongoing trials evaluating ruxolitinib combinations in patients with myelofibrosis.

Partner drug class	Specific agent	Clinicaltrials.gov identifier
Histone deacetylase inhibitor	Panobinostat	NCT01693601
	Panobinostat	NCT01433445
	Pracinostat	NCT02267278
Phosphatidylinositol-3-kinase (delta isoform) inhibitor	INCB050465	NCT02718300
	Idelalisib	NCT02436135
	TGR1202	NCT02493530
Immunomodulatory agent	Thalidomide	NCT03069326
	Lenalidomide	NCT01375140
	Pomalidomide	NCT01644110
Janus kinase 1 inhibitor	Itacitinib	NCT03144687
BH3-mimetic	Navitoclax	NCT03222609
Hedgehog (smoothened) inhibitor	Vismodegib	NCT02593760
	Sonidegib	NCT01787552
Cyclin-dependent kinase 4/6 inhibitor and PIM kinase inhibitor	Ribociclib and PIM447	NCT02370706
Androgen	Danazol	NCT01732445
Interferon	Pegylated interferon alfa 2a	NCT02742324
Hypomethylating agent	Azacitidine	NCT01787487
Activin receptor ligand trap	Sotatercept	NCT01712308
	Luspatercept	NCT03194542
Erythropoiesis stimulating agent	Any (observational study)	NCT03208803